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The vast majority of potent tyrosine kinase-specific inhibitors described to date are competitive with ATP. Although these species tend to exhibit impressive Ki values, their effectiveness under physiological conditions is markedly impaired due to high intracellular concentrations of ATP. Peptide-based inhibitors that are competitive with protein/peptide substrate have been described for various tyrosine kinases, however these nonphosphorylatable phenylalanine-containing inhibitors typically display poor inhibitory profiles. We have found that L-Dopa serves as a powerful nonphosphorylatable tyrosine mimetic. In addition, we have identified several promising leads in the creation of nonpeptidic inhibitors of tyrosine kinases. Since these species are not directed to the ATP binding region, they do not suffer from the disadvantages associated with ATP analogs.

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A M Edelman 9/25/97

PI - Signature Date

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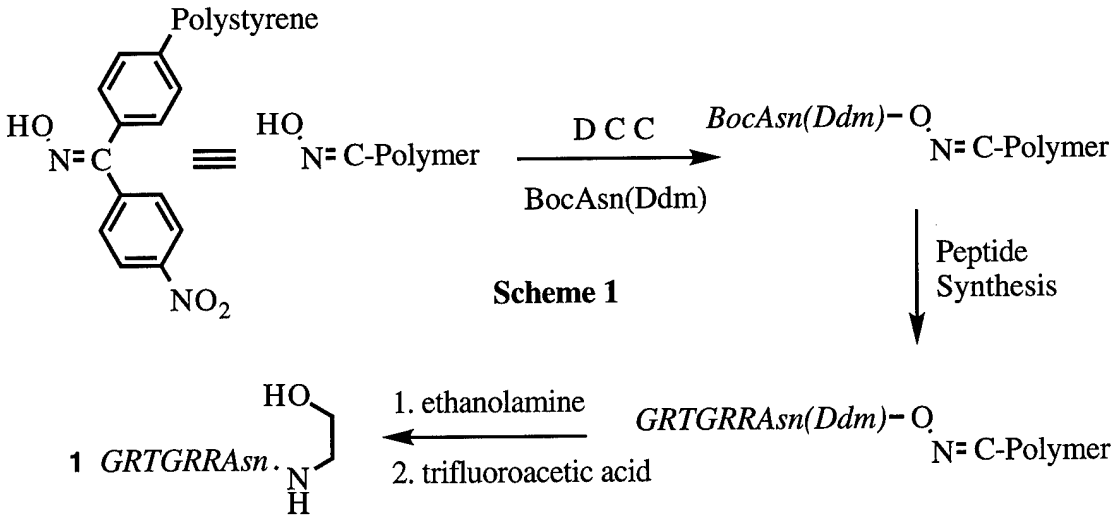
## Introduction

Protein kinases serve as key components along the signal transduction pathways leading to cell division. Numerous studies have now confirmed that constituitively active protein kinases (arising from genetic mutation) are often responsible for uncontrolled cell growth. A dramatic case in point are the erb B gene product, which is overexpressed in nearly 30% of all breast cancer tissue. Furthermore, overexpression of this tyrosine-specific protein kinases has been directly correlated with a poor prognosis in terms of relapse and survival rates. Furthermore, it has now been demonstrated that interference with the catalytic activity of this and related enzymes results in the restoration of normal growth to cancerous cells. In short, protein kinase inhibitors may constitute an entirely novel class of chemotherapeutic drugs, agents which do not kill cancer cells, but rather serve to halt the uncontrolled cell growth that is responsible for the symptoms associated with cancer. While this area is ripe for exploration, the creation of specific protein kinase inhibitors has been painfully slow. We have recently developed a novel chemical methodology for the rapid surveillance of the active site structure and specificity of protein kinases. Most importantly, this strategy has enabled us to acquire data that highlights both the similarities and differences among these family members in the context of inhibitor design. Until recently, our work had been limited to the serine/threonine-specific protein kinases (*vide infra*). However, with the acquisition of the Army breast cancer funds, we have now begun to explore the active site substrate specificity behavior of the tyrosine-specific protein kinases, of which erb B is a member. Our initial studies have focused on the active site substrate specificity of c-Src and v-abl kinases as well as a detailed examination of the kinetic mechanism of the v-fps-catalyzed phosphorylation of peptide substrates. During the past year we have begun to explore the ability of tyrosine protein kinases to accommodate nonphosphorylatable tyrosine mimetics within their active site regions. As we have previously seen with the active site *substrate* specificity of these enzymes, the active site inhibitor specificity differs, in a dramatic fashion, among "tyrosine-specific" protein kinases. In addition, we have developed a novel chemical library protocol that should eventually lead to nonpeptidic inhibitory species that are directed to the protein binding site of these enzymes.

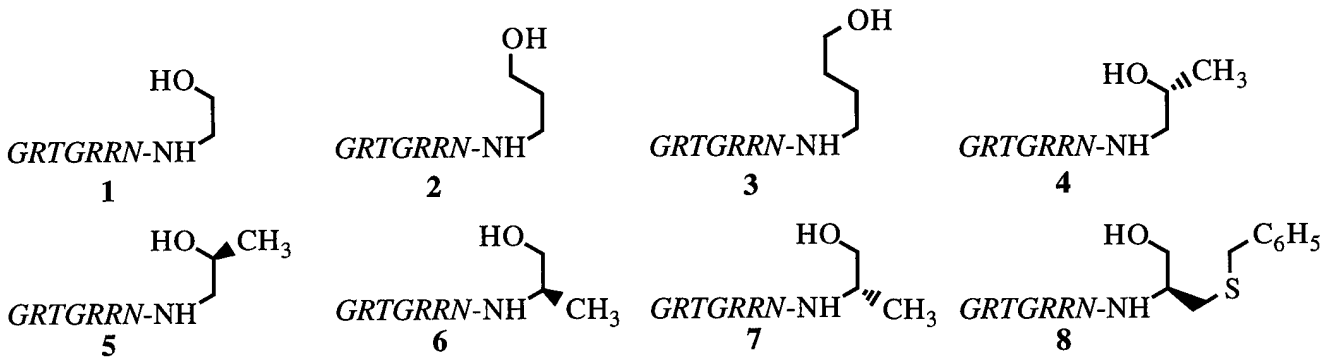
Until recently, only peptides containing Ser,[1] Thr,[1] hydroxyPro,[2] and homoSer[3] had been identified as PKA substrates. An analysis of substrate specificity serves as a crucial prerequisite for the design of novel inhibitors containing elaborate functionality. Unfortunately, detailed information on the active site substrate specificity of protein kinases is not available due to synthetic obstacles associated with the preparation of peptide-based substrates and inhibitors that contain hypermodified residues. We have circumvented these difficulties by inserting the residue of interest into the active site-directed peptide after solid phase peptide synthesis (SPPS). We prepared the peptide Gly-Arg-Thr-Gly-Arg-Arg-Asn on an oxime resin.[4] The sequence is based on the portion of a naturally occurring inhibitor that binds just to the N-terminal side of the PKA phosphorylation site. After SPPS is complete, the peptide is displaced from the resin employing an alcohol-containing amine (shown for ethanolamine; **1** in Scheme 1). The key advantage associated with this protocol is that the hypermodified amino acid residue can be incorporated into the peptide without exposing the residue to the harsh conditions of SPPS. Furthermore we have found that Gly-Arg-Thr-Gly-Arg-Arg-Asn-oxime resin can be synthesized without Arg and Thr side chain protecting groups, thereby circumventing the need for the final HF deprotection step (the dimethoxydiphenylmethyl (Ddm) group is removed with CF3COOH).

The active site substrate specificity of PKA includes the following key features: (1) *An achiral residue at the phosphorylation site will serve as an efficient substrate.* Compound **1** serves as a surprisingly efficient PKA substrate ($K_m$ = 114 μM and $V_{max}$ = 8.3 μmol/min-mg). Similarly, the propyl alcohol side chain (**2**), also serves as a substrate, however with a higher $K_m$ (525 μM) and lower $V_{max}$ (4.8 ± μmol/min-mg). PKA phosphorylates the butyl alcohol side chain of **3**, albeit relatively




**Scheme 1**

inefficiently ($K_m$ = 794 μM and $V_{max}$ = 0.175 μmol/min-mg). Exceedingly simple derivatives of ethanolamine can now be constructed with unusual functionality (transition state analogs, mechanism-based inhibitors, etc.), thereby mitigating the need for difficult-to-prepare chiral amino acid derivatives (*vide infra*). (2) *The side chain ß-configuration recognition element.* PKA phosphorylates the



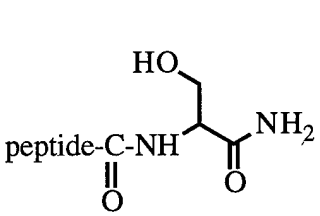

secondary alcohol of Thr, however it is not known if the absolute configuration at this center is crucial. Compound **4** contains the same configuration present in the ß-carbon of Thr. This species is a respectable substrate ($K_m$ = 607 μM and $V_{max}$ = 1.24 μmol/min-mg), exhibiting a $K_m$ that is 5-fold higher and a $V_{max}$ that is 7-fold lower than those displayed by compound **1**. Our analysis of PKA active site structure suggests that Phe187 may interfere with the phosphorylation of alcohols that contain ß-substituents. Of greater significance is that inversion of configuration at the ß-center (i.e. **5**) blocks phosphoryl transfer to the alcohol-bearing compound. Active site residues Ser53 and Phe54 appear to sterically preclude binding of the latter configurational isomer. Consequently, if a branched ß-carbon is required for inhibitor design, the stereochemistry at the ß site appears to be critical. (3) *The side chain α-configuration of the substrate is an important recognition element.* Compound **6**, which contains the same α-configuration present in L-Ser, is an excellent substrate ($K_m$ = 35.0 μM and $V_{max}$ = 9.73 μmol/min-mg). In contrast, **7** (corresponding to D-Ser) fails to serve as a substrate. Analogous results were noted with other residues bearing the inverted stereochemistry at this position.[5] These results are reassuring since D-Ser, in an active site-directed peptide, also fails to serve as a PKA substrate. This indicates that the C-terminal truncated peptides under evaluation in this study conform to the same specificity requirements exhibited by their larger counterparts. Thr201, which lies just below the phosphorylation site, appears to sterically prevent residues containing the latter configuration from serving as phosphoryl acceptor moieties. (4) *Substituents at the α-position alter the efficacy of phosphoryl transfer.* The residue just to the C-terminal side of the Ser moiety in PKA substrates is accommodated in a hydrophobic pocket located within the

general region of the active site. Consequently, a lipophilic substituent off the α-carbon center may improve the kinetics of kinase-catalyzed phosphoryl transfer. A number of peptides bearing hydrophobic substituents at this position were investigated as PKA substrates.[5] We found that **8** is a remarkably effective substrate, with $K_m$ (7.17 μM) and $V_{max}$ (20.7 μmol/min-mg) values that compare favorably with those ($K_m$ = 16 μM and $V_{max}$ = 20 μM) exhibited by Kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly).[1]

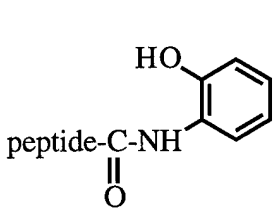
In contrast to the active site behavior exhibited by PKA, the cGMP-dependent protein kinase ("PKG") utilizes both configurational isomers at the α- as well as the ß-stereocenters as substrates.[6] In other words, both **4** and **5** as well as **6** and **7** are phosphorylated by PKG. Based upon these results, we devised an affinity label that is absolutely specific for PKG.[7] In addition, we constructed several reversible inhibitors that likewise exhibit a strong selectivity for PKG.[8] We note as an aside that a recently published Protein Kinase FactsBook pointed out the difficulty of selectively inhibiting PKG in the presence of PKA. In short, the inhibitors described in references 7 and 8 are the first examples of PKG-selective inhibitors.

PKA phosphorylates both Ser and Thr residues at Xaa in the active site-directed peptide Leu-Arg-Arg-Ala-Xaa-Leu-Gly.[1] In contrast, the corresponding Tyr-containing peptide fails to serve as a substrate.[9] These results are representative of the absolute substrate specificity that is attributed to the vast majority of protein kinases (for apparent exceptions, see reference 10). There are a number of structural differences between Tyr and the Ser/Thr pair; any one of which may be exploited by PKA to ensure strict active site substrate specificity. Perhaps the most obvious difference between these residues is the relative position of the hydroxyl moiety, which is clearly further from the peptide backbone in Tyr than in Ser or Thr. In the case of peptide **14**, the aromatic alcohol is aligned in approximately the same fashion as the aliphatic alcohols in the Ser/Thr pair. In contrast, the relative geometries and distances between the hydroxyl moiety and backbone peptide bond in **15** and **16** (substitution pattern similar to Tyr) are distinct from those in Ser/Thr and **14**.

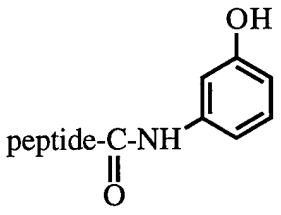
Peptides **14**-**16** were incubated with PKA, however only **14** was phosphorylated to any significant extent.[11] These results are consistent with the notion that the position of the aromatic hydroxyl group relative to the peptide backbone is crucial for PKA-catalyzed phosphoryl transfer. The $K_m$ for **14** (793 μM) is similar to that reported for the ß-substituted-containing species **4**. In contrast, the $V_{max}$ (68 nmol/min-mg) is significantly lower than those previously found for aliphatic alcohols.[11] Graves has noted that the $V_{max}$ values associated with protein kinase-catalyzed Tyr phosphorylations are substantially smaller than those obtained from kinase-catalyzed Ser phosphorylations and has proposed that this behavior is due to the reduced nucleophilicity associated with the aromatic alkoxide.[12] Nevertheless, it is noteworthy that the $V_{max}$ exhibited by **14** compares favorably with $V_{max}$ values reported for Tyr kinase-catalyzed reactions. For example, substrates for the insulin receptor kinase exhibit values from 6 to 72 nmol/min-mg.[13-14] We subsequently prepared the electron rich aromatic alcohols **17** and **18**, species



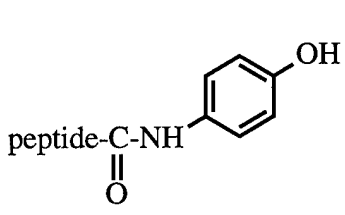

**14**



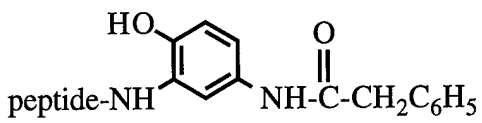

**15**




**16**

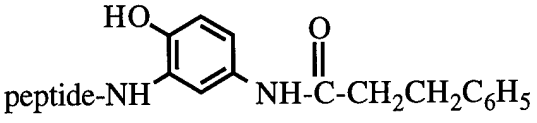



which should exhibit enhanced nucleophilicity relative to **14**. Indeed, the $V_{max}$ values for **17** and **18** approach those previously obtained for secondary alcohols (approximately 1 μmol/min-mg) and, moreover, the $K_m$ values are nearly an order of magnitude lower.[15]




**17**

($K_m$ = 81 μM; $V_{max}$ = 920 nmol/min-mg)




**18**

($K_m$ = 57 μM ; $V_{max}$ = 890 nmol/min-mg)

Interestingly, although PKG and PKA exhibit comparable specificity patterns toward **14-16**, PKC differs in a dramatic fashion by utilizing all three peptides as substrates. These results are not only another example of the differing active site specificities that closely related kinases display toward unnatural residues, but also represents the first example of alcohol-bearing species (i.e. **15** and **16**) that exhibit an absolute specificity for a mitogenic protein kinase. These results should have important implications with respect to the design of inhibitors that specifically target kinases implicated in the signaling pathways responsible for cell division.

As we found for the serine/threonine-specific protein kinases, tyrosine-specific protein kinases will likewise recognize a wide variety of alcohol-containing residues as substrates.[16] Furthermore, we have also demonstrated that the abl and src kinases exhibit differing active site specificities toward these unnatural residues.[17] We now report that this specificity is not just limited to substrates but includes inhibitory functionality as well. Furthermore, we describe our first tentative steps toward the construction of nonpeptidic inhibitors of tyrosine kinases, species that may ultimately provide an entry into medicinally useful agents.

## Results (10-1-96 through 9-31-97 )

Tyrosine-specific protein kinases play a key role in transducing growth promoting signals from the cell surface to the nucleus. Consequently, it is not surprising that there has been, and continues to be, a profound interest in creating inhibitors that precisely target specific members of this enzyme family. The most powerful inhibitors described to date are targeted to the ATP binding site.[18] Unfortunately, the inhibitory effectiveness of these species is markedly impaired under physiological conditions due to the high intracellular concentrations of ATP.[19] Peptide-based inhibitors, entities competitive with protein substrate binding, have been described but are generally disappointing in terms of inhibitory potency with $K_i$ values generally in the 1 - 2 mM range.[20] The latter species are typically generated by replacing the phosphorylatable tyrosine residue in an active site-directed peptide with a nonphosphorylatable phenylalanine moiety.[21] The discouraging inhibitory profiles of these phenylalanine-based peptides may be attributable to the missing aromatic hydroxyl functionality, which likely engages in productive active site interactions when present in the corresponding tyrosine-containing substrate. In addition, several recent studies have demonstrated that the $K_m$ values exhibited by some peptide substrates of protein kinases exaggerate how well these peptides actually bind to the target kinase.[3a,22] Although these explanations may provide a rationale for the weak activity of phenylalanine-based peptides, the poor inhibitory behavior of these species nonetheless demonstrate the need for nonphosphorylatable tyrosine analogs that dramatically enhance enzyme affinity.

The synthesis of peptides containing unnatural and potentially inhibitory residues can be time-consuming and, in many instances, challenging.[23] Fortunately, these synthetic obstacles can be circumvented by directly attaching the residues of interest to the N- or C-terminus of an active site-directed peptide.[24] The latter approach provides a rapid and efficient means to acquire peptides containing a structurally diverse ensemble of functionality specifically targeted for active site insertion. The general synthetic approach is illustrated in Fig. 1. We recently constructed a peptide-based library containing an

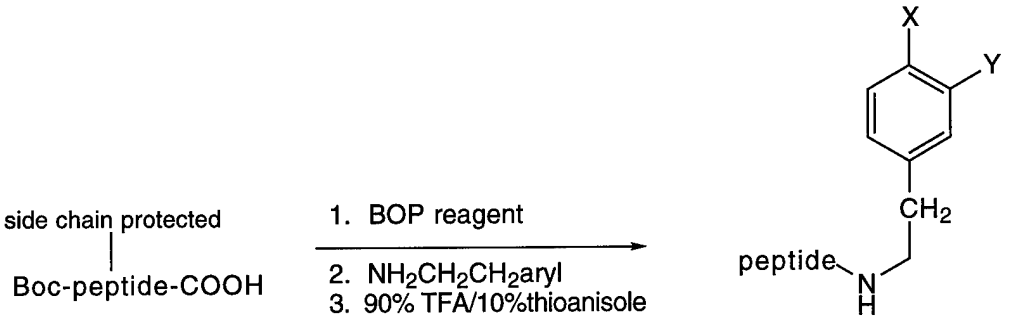



**Figure 1**

**19, 23** X, Y = H
**20, 21** X, Y = OH
**22** X = OH; Y = H

array of C-terminus-linked phenethylamine analogs in which the phosphorylatable *para*-substituted hydroxyl of tyramine was replaced with an assortment of nonphosphorylatable functionality.[24] Although a few functionality, such as sulfonamide, do enhance enzyme affinity without concomitant phosphorylation, the overall inhibitory potency of this family of peptides is disappointingly modest. Clearly, tyrosine-specific protein kinases have evolved to coordinate an aromatic hydroxyl moiety within the active site region. Is it possible to retain the alcohol of tyrosine, yet affix additional functionality to the aromatic nucleus that not only precludes phosphoryl transfer but actually enhances enzyme affinity? We decided at the outset not to employ sterically demanding substituents *ortho* to the alcohol moiety since we were concerned that these types of substitution patterns might interfere with the ability of the aromatic hydroxyl group to engage in productive hydrogen bonding interactions with active site residues. We now report that dopamine, and its corresponding amino acid, L-Dopa, serve as potent nonphosphorylatable mimetics of tyrosine in $pp60^{c\text{-}src}$-targeted peptides.

*A dopamine-substituted peptide is a significantly more potent inhibitor of $pp60^{c\text{-}src}$ than the corresponding phenethylamine derivative.* The phenethylamine-based peptide (**19**) and its dihydroxy-counterpart (**20**) were prepared according to the synthetic scheme outlined in Fig. 1.[25] The dopamine-derivative **20** is 31-fold more effective as an inhibitor of $pp60^{c\text{-}src}$ than peptide **19**.

*A dopamine-substituted peptide is not a $pp60^{c\text{-}src}$ substrate.* Protein kinases are commonly assayed for activity by quantitating the incorporation of $^{32}P$ (from $^{32}P$-$\gamma$[ATP]) into serine, threonine, and/or tyrosine residues positioned within positively charged active site-directed peptides.[24] The latter coordinate to a negatively charged phosphocellulose disk, which can be subsequently counted for radioactivity. Since **20** lacks the primary sequence necessary for binding to phosphocellulose disks, we prepared an arginine-substituted derivative of **20** (Arg-Arg-Arg-Arg-Arg-Leu-Glu-Glu-Leu-Glu-dopamine; **21**). The substrate efficacy of **21** was then compared with that of the tyramine-based analog **22**, a known a $pp60^{c\text{-}src}$ substrate.[26] In addition, we prepared the nonphosphorylatable phenethylamine-based peptide, Arg-Arg-Arg-Arg-Arg-Leu-Glu-Glu-Leu-Glu-phenethylamine (**23**), as a control. As is apparent from Fig. 2, peptide **21** is inert under conditions in which **22** suffers phosphorylation.

*An L-Dopa-substituted peptide is a significantly more potent inhibitor of $pp60^{c\text{-}src}$ than the corresponding phenylalanine derivative.* Do the inhibitory trends observed with C-terminus-substituted peptides (i.e. **1** and **2**) hold in more conventional peptidic environments? In order to address this question we prepared the peptides Glu-Glu-Leu-Leu-**Phe**-Gly-Glu-Ile (**24**) and Glu-Glu-Leu-Leu-**Dopa**-Gly-Glu-

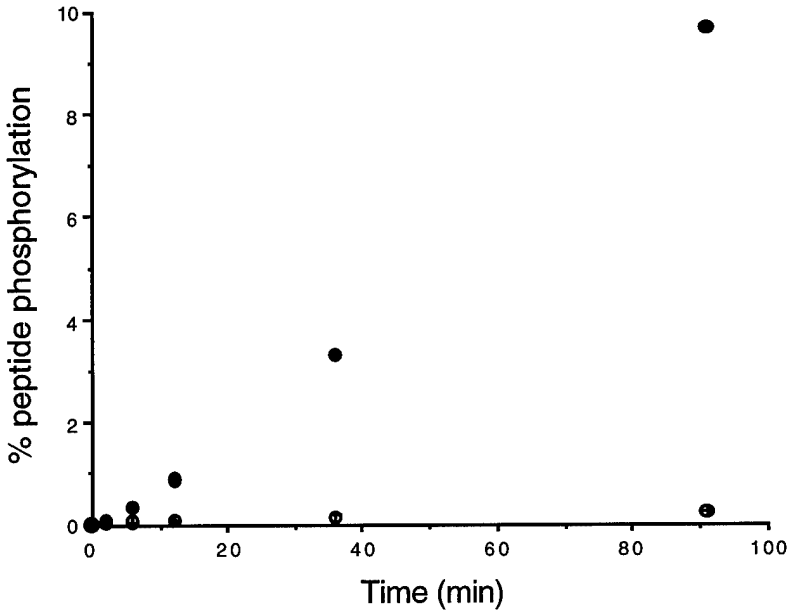



**Figure 2.** pp60c-src-catalyzed phosphorylation of **21** (+), **22** (•), and **23** (o) as a function of time. After 16 h, 50% of **22** underwent phosphorylation, whereas less than 1% of **21** or **23** is $^{32}$P-labeled under identical conditions (the 16 h data points are not shown). Since **5** lacks a hydroxyl moiety, it is unlikely that the trace levels of radioactivity associated with **3** and **5** are due to phosphorylation.

Ile (**25**). The primary sequence encompassing the Phe and Dopa residues was chosen, in part, from the results of a previous study using a combinatorial peptide library to assess pp60$^{c\text{-}src}$-specificity.[27] As is evident from Table 1, the inhibitory trend between **19** and **20** holds for the conventional peptide dyad **24**

| Inhibitor | $IC_{50}$ (μM) | $K_i$ (μM) |
|---|---|---|
| Glu-Glu-Glu-Glu-**phenethylamine** (**19**) | 1650 ± 10 | – |
| Glu-Glu-Glu-Glu-**dopamine** (**20**) | 53 ± 2 | – |
| Glu-Glu-Leu-Leu-**Phe**-Gly-Glu-Ile (**24**) | 950 ± 25 | 860 ± 20 |
| Glu-Glu-Leu-Leu-**Dopa**-Gly-Glu-Ile (**25**) | 29 ± 2 | 16 ± 3 |

**Table 1**

and **25**. In the latter case, **25** is a 33-fold more effective inhibitor than **24**. Furthermore, both peptides are competitive inhibitors versus peptide substrate (Figs 3). Indeed, the difference in $K_i$ values exhibited

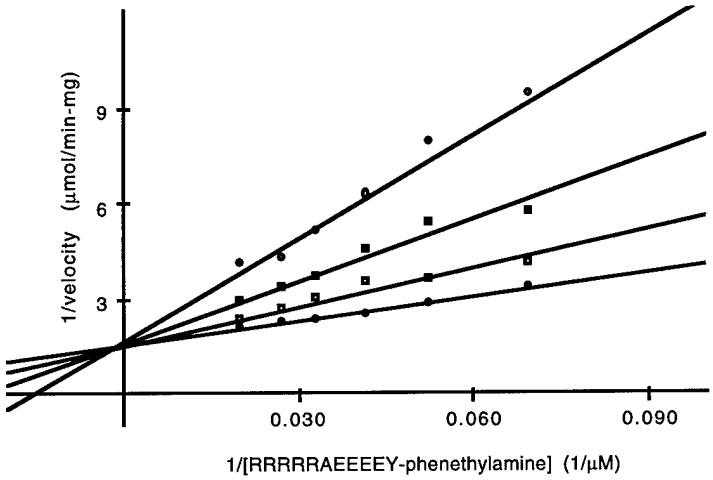



**Figure 3.** The Lineweaver-Burk double reciprocal plot of the L-Dopa-containing peptide **25** as a function of varied peptide substrate.

by **24** and **25** is even more substantial (55-fold) than that observed for the corresponding $IC_{50}$s. Finally, since the Dopa-containing peptide serves as a *non*competitive inhibitor versus variable ATP ($K_i$ = 14 ± 2 µM; see Fig 4), it is evident that this inhibitory species does not coordinate to the ATP binding site.

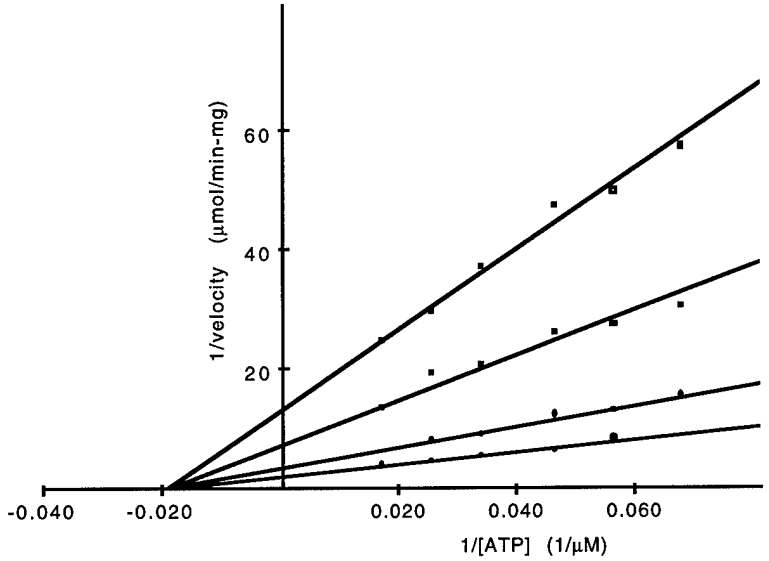



**Figure 4.** The Lineweaver-Burk double reciprocal plot of the L-Dopa-containing peptide **25** as a function of varied ATP.

*The L-Dopa peptide* **25** *only serves as a reversible inhibitor of pp60$^{c\text{-}src}$.* L-Dopa is best known as a medicinal agent for the treatment of Parkinson's disease.[28] However, this amino acid has also been found in proteins. Dopa plays a key role in protein cross-linking in invertebrates (e.g. byssal adhesion of marine mussels) via oxidation to the corresponding *ortho* quinone.[29] As a result, we were somewhat concerned that fortuitous oxidation of the L-Dopa residue might be responsible for the impressive inhibitory profile of **25**. However, we failed to detect a time-dependent inactivation of pp60$^{c\text{-}src}$ in the presence of **25** that is any more substantial than in the absence of the peptide (i.e. a slight, yet identical, loss in tyrosine kinase activity as a function of time is observed in both the presence and absence of **25**; Fig. 5). Consequently, we conclude that the Dopa-containing peptide only serves as a simple reversible inhibitor of pp60$^{c\text{-}src}$.

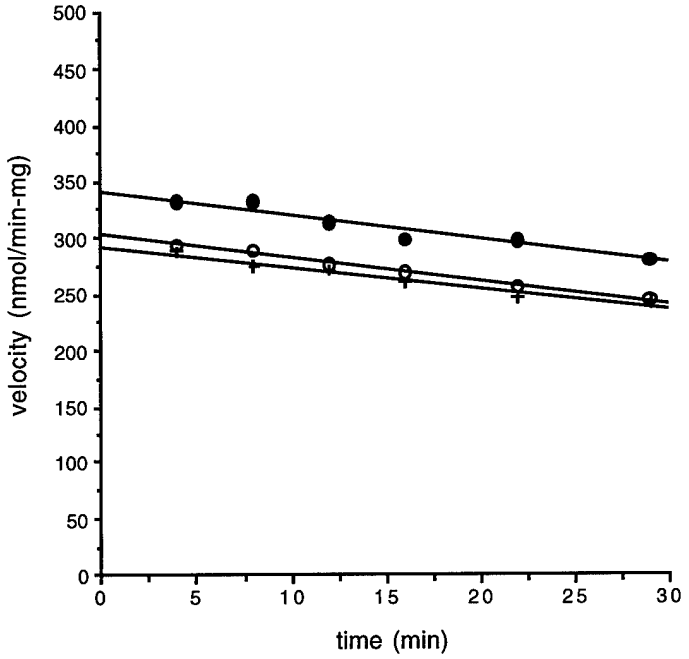
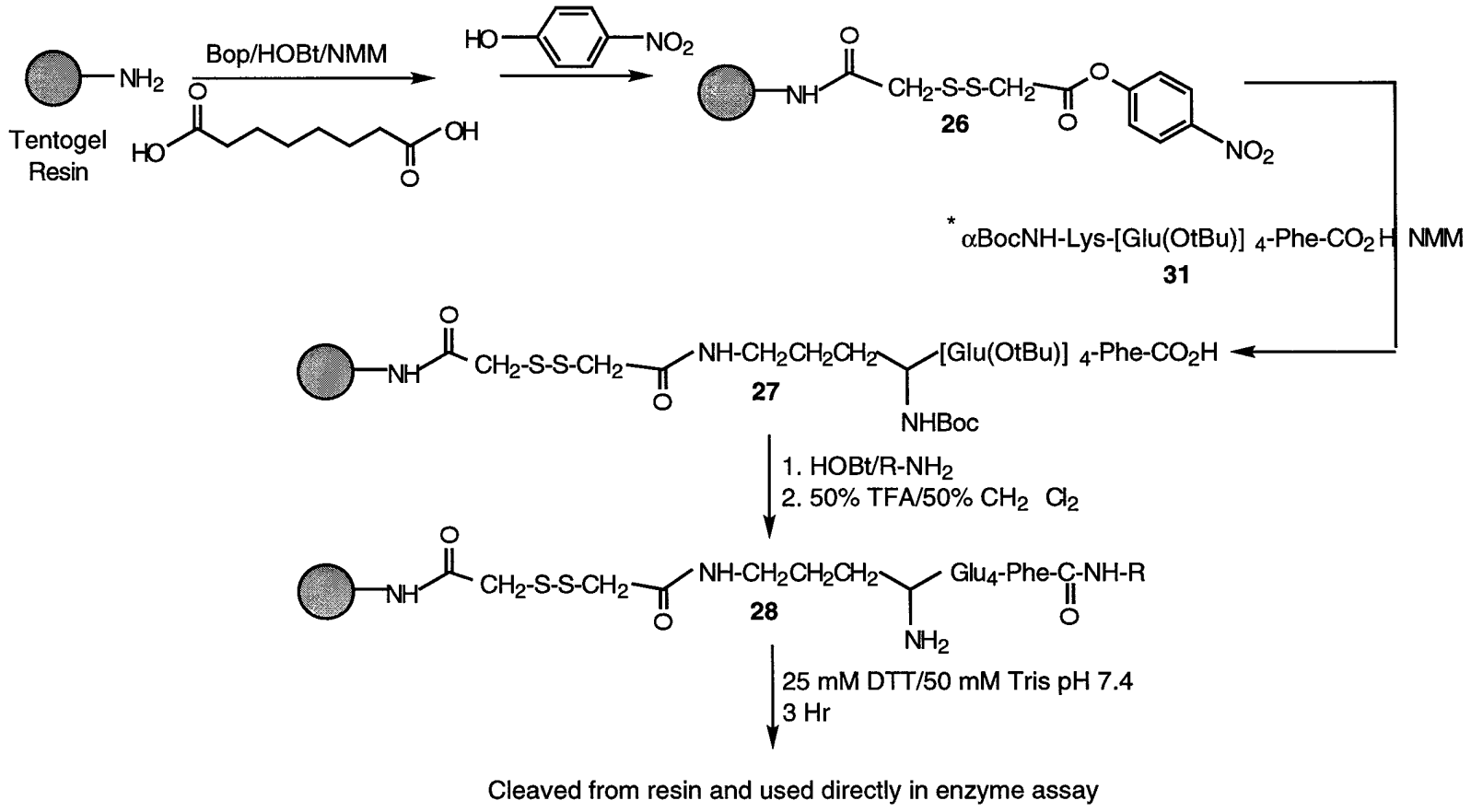


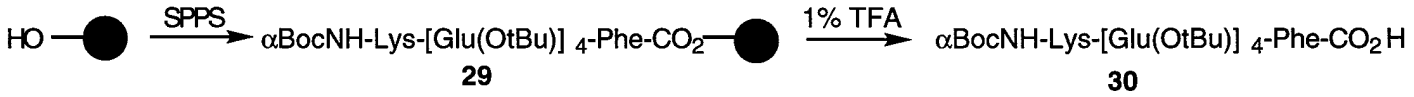

**Figure 5.** The time-dependent activity of pp60c-src in the absence of peptide inhibitor (•), in the presence of 900 μM **24** (+), or 30 μM **25** (o). Concentrations chosen are near the $IC_{50}$ values of the respective inhibitors. The overall lower activity of the enzyme in the presence of the inhibitory peptides is likely due to reversible binding (only a five-fold dilution from incubation solution to assay solution was employed).

The most potent tyrosine kinase-specific inhibitors reported to date are species that coordinate to the ATP-binding site. Although the $K_i$ values associated with these inhibitory species are in the nanomolar range, micromolar concentrations will be required to overcome the high intracellular concentrations of ATP present in most cell types. Furthermore, it is important to be cognizant of the fact that there are a large number of ATP utilizing enzymes present in mammalian cells. Although peptide-based inhibitors do suffer from the bioavailability point of view, their peptidomimetic counterparts may ultimately offer greater opportunities for creating inhibitors that can exquisitely discriminate between closely related kinases. Until recently, the inhibitory potency of tyrosine kinase-targeted peptides has been, in general, extremely disappointing. With the advent of nonphosphorylatable tyrosine mimetics, such as L-Dopa, peptide-based

## Scheme II



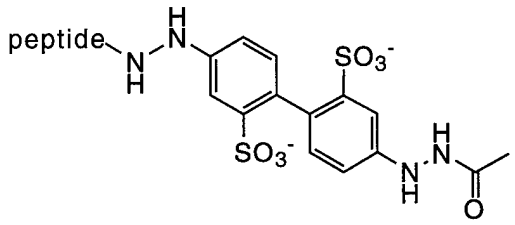

* synthesized as follows (where • = methoxy alkoxy benzylalcohol resin):



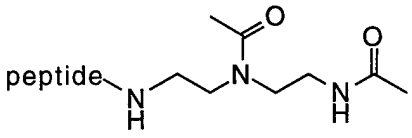
species and their cognates can now be given serious consideration as potentially useful inhibitors for members of the tyrosine-specific subfamily of protein kinases.

We recently initiated a library approach that, in combination with the work described above, should lead to identification of nonpeptidic inhibitors of tyrosine-specific protein kinases. We have artificially divided the active site-directed peptide substrates and inhibitors of tyrosine kinases into three separate regions: (a) the phosphoryl acceptor region, (b) The N-terminal and (c) C-terminal regions. In the work described above, we focused on the acquisition of a tyrosine replacement for (a). We have now examined nonpeptidic substitutes for the C- and N-terminal regions. For our initial studies we used phenylalanine as the tyrosine surrogate (since the tyrosine mimetic studies had just commenced when we initiated the library project). The synthetic approach that we devised for this library is illustrated in scheme II.

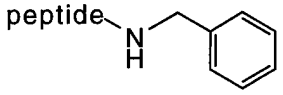
We screened more than 50 peptide-nonpeptide hybrids containing different nonpeptidic appendages attached to the C-terminus of the active site directed peptide **28**. Three lead appendages are **32-34**. We




**32**

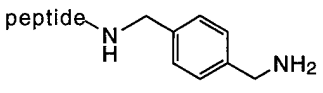



**33**

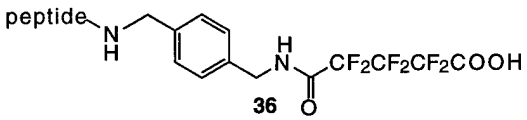



**34**

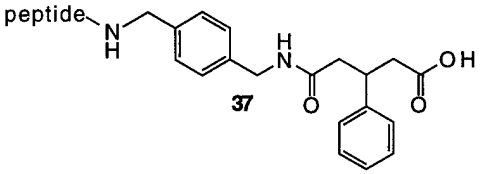
decided that **34** provided the best opportunities for subsequent structural elaboration and therefore synthesized **35** and modified the free benzylic amine with a variety of activated carboxylic acids. Several lead compounds were identified (e.g. **36-37**).




**35**

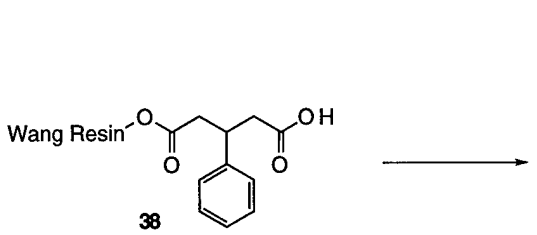



**36**

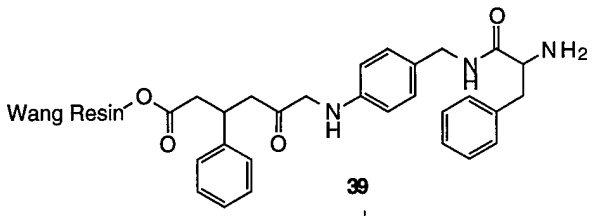



**37**

The aquisition of nonpeptidic inhibitors was based on species **37**. We prepared the analog **38** via

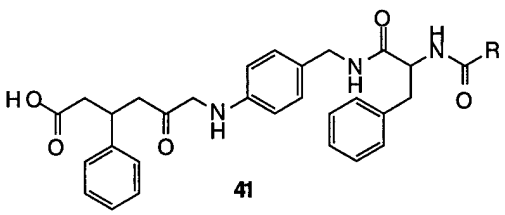



**38**

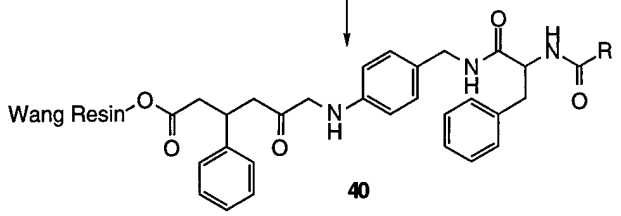



**39**

**Scheme III**

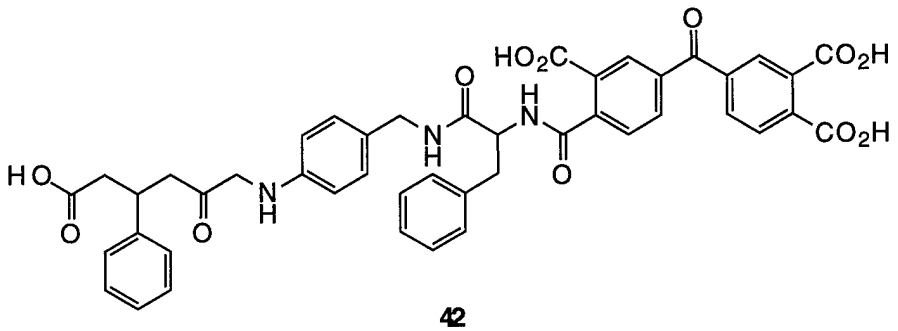



**41**

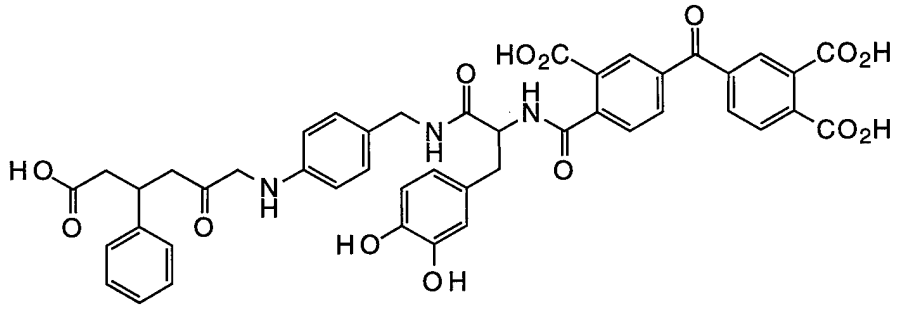



**40**

the strategy outlined in scheme III, and subsequently modified the free amine with a number of activated carboxylic acids. The lead compound that has emerged from these experiments is **42**. Although the $K_i$ is



**42**



**43**

approximately 250 μM, this value is better than the best phenylalanine-based *peptide inhibitors* that have ever been reported for pp60$^{c\text{-}src}$ (e.g. note the $IC_{50}$ for peptide **24** in Table 1). We now plan to synthesize and test the L-Dopa-containing analog **43**. If the trend shown in Table 1 holds, then the $K_i$ for this species should be submicromolar. These results represent the first effort ever to move from the peptide to the nonpeptide world in the design of effective tyrosine kinase inhibitors. As we have noted above, as well as in a recent review[30], inhibitors directed to regions other than the ATP binding site hold decided advantages over their ATP analog counterparts.

## Conclusions

The vast majority of potent tyrosine kinase-specific inhibitors described to date are competitive with ATP. Although these species tend to exhibit impressive $K_i$ values, their effectiveness under physiological conditions is markedly impaired due to high intracellular concentrations of ATP. Peptide-based inhibitors that are competitive with protein/peptide substrate have been described for various tyrosine kinases, however these nonphosphorylatable phenylalanine-containing inhibitors typically display poor inhibitory profiles. We have found that L-Dopa serves as a powerful nonphosphorylatable tyrosine mimetic. In addition, we have identified several promising leads in the creation of nonpeptidic inhibitors of tyrosine kinases. Since these species are not directed to the ATP binding region, they do not suffer from the disadvantages associated with ATP analogs.

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$$\text{equation (1)}\quad \frac{v_i}{v} = \frac{K_m + [\text{ATP}]}{K_m(1+\frac{[\text{I}]}{K_\text{i}}) + [\text{ATP}]} \qquad \text{equation (2)}\quad \frac{v_i}{v} = \frac{5}{.005(1+\frac{[\text{I}]}{K_\text{i}}) + 5}$$

be approximately 1,000-fold greater than its experimentally derived $K_i$ value. Although the affinities of the most potent PTK inhibitors are in the nanomolar range, their effectiveness under physiological conditions should be decidedly micromolar. In contrast, the concentration that generates a 50% reduction in activity for inhibitors that are noncompetitive with respect to ATP [$v_i/v = (K_i/(K_i + [I])$] is equivalent to $K_i$.


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1. Jinkui Niu and David S. Lawrence "Nonphosphorylatable Tyrosine Surrogates: Implications for Protein Kinase Inhibitor Design" *J. Biol. Chem.*, **1997**, *272*, 1493-1499.

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